

Zürich 1982

DNA of Bovine Herpesvirus Type 1 in the Trigeminal Ganglia of Latently Infected Calves

M. Ackermann, Dipl vet; E. Peterhans, Dr med vet; R. Wyler, Dr med vet

Discretionary
vet. med. Fakultät
Bibliothek Prof. Dr. R. Wyler
Korrespondenz: Prof. Dr. E. Peterhans

SUMMARY

Twelve calves infected with bovine herpesvirus type 1 (BHV-1) were killed when in a latent state of infection. Latency was verified 30 days after virus inoculation of the calves by seroconversion, absence of virus shedding, and in 2 calves, by recrudescence of the infection after they were treated with dexamethasone.

By in situ hybridization techniques and autoradiography, DNA of BHV-1 was detected in 13 of 23 trigeminal ganglia of latently infected calves. Viral DNA was restricted to the nucleus of nerve cells. Single neurons harboring BHV-1 DNA were observed in 4.9% of the sections ($n = 325$) of the trigeminal ganglia. The results obtained correspond to those known from herpes simplex virus infections in mice.

The implications for the virus-host relationship are discussed.

Infectious bovine rhinotracheitis (IBR) was first observed in Colorado in 1950¹ and was shown to be caused by bovine herpesvirus type 1 (BHV-1 = IBR virus).² The signs of IBR are fever with rhinotracheitis, conjunctivitis, and encephalitis.³⁻⁵ The severity of these depends on the virus strain and on the age of the animal affected—encephalitis and death generally being restricted to calves. In addition, there may be abortion in the last 3 months of pregnancy.⁶

Animals having recovered from IBR may show signs of IBR repeatedly without being exposed to reinfection.⁷ This indicates that the virus can stay in its host in a latent form and may be reactivated even after long periods. This suggestion is supported by the finding that virus shedding with or without signs of IBR can be provoked by dexamethasone (DM) treatment.⁸ Thus, the situation seems to be similar to that induced by herpes simplex virus type 1 (HSV-1), which causes recurrent episodes of disease in persons and mice.⁹⁻¹¹ Indeed, it has been shown by immunofluorescent methods that BHV-1, like HSV-1, travels from the site of primary infection to the trigeminal ganglion along the axons of sensitive nerve cells. The viral antigen can then no longer be detected in the ganglion. Shortly before the disease

becomes apparent again, viral replication takes place in the ganglion.¹²⁻¹⁴ Infective virus can also be recovered from trigeminal ganglia of clinically healthy cattle by cocultivation with susceptible cells.¹⁵ This indicates that the trigeminal ganglion may be the site of latent infection in IBR and that the virus may persist in the ganglion in the form of DNA.

Using in situ hybridization and autoradiography, we now show that the DNA of BHV-1 is present in the nucleus of ganglion cells during latency.

Materials and Methods

Virus—The Los Angeles strain of IBR virus (IBR-LA) was obtained from Prof. Witzigmann, München, Germany. The virus was serially passaged 4 to 5 times in a bovine kidney cell line (MDBK). The infectivity titer ranged between 10^8 and $10^{8.3}$ median tissue culture infective doses (TCID₅₀)/ml.

Cell Culture Methods—The MDBK cell cultures were grown in RPMI 1640 medium supplemented with 10% bovine fetal serum (BFS) and antibiotics (100 U of penicillin and 100 µg of streptomycin sulfate/ml). The medium was buffered with 30 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES). Cultures were incubated at 37°C. Primary bovine embryo lung cell cultures (eBLC) were grown in Eagle's minimal essential medium (EMEM) supplemented as were MDBK cultures.

Infection of Animals—Ten Swiss Braunvieh and 2 Friesian female calves (No. 1 through 12) were inoculated at 1 to 2 months of age with IBR-LA virus by the tracheal route (10 ml containing 10^7 TCID₅₀).

Noninfected Control Animals—Six randomly selected Swiss Braunvieh calves slaughtered at the age of 6 months served as noninfected controls (1N through 6N). On the day of slaughter, these animals did not excrete IBR virus and showed no antibodies to IBR virus.

Dexamethasone Treatment—Calves 11 and 12 were given DM^a iv (0.1 mg/kg of body weight) between postinoculation day (PID) 55 and 59.

Sample Collection—The nasal and ocular secretions from calves 1 through 12 were obtained by swab technique on the day before IBR-LA virus was inoculated and every 2nd PID until PID 23. Thereafter, swabs were taken weekly and on the day of slaughtering. In addition, swabs from calves 11 and 12 were taken daily during the period of treatment with DM and 2 weeks thereafter. From that point on, sample collections were done once a week and on the day of slaughtering (see Table 1).

Virus Detection in Swabs—Swabs were squeezed out imme-

^a Voren, Boehringer, Ingelheim, Germany.

Received for publication Apr 24, 1981.

From the Institute of Virology, University of Zürich CH-8057, Switzerland.

The authors thank Drs. Monika Engels, P. Ruesch, B. Hauser, D. Schümperli, T. Schmidt, H. J. Rziha, P. Wild, and H. Matile for help and encouraging discussions; F. Schwendimann, Elisabeth Wenk, and Edith Gubler for technical assistance; and Dr. Margaret Chipchase and Christina Gerber for manuscript preparation.

1982

diately after collection into 2 ml of EMEM. Virus detection was performed by inoculating eBLC cultures and examining the cell layer for characteristic cytopathic effects (CPE) during 10 days. Two blind passages were performed from negative samples.

Virus Purification—Purification of IBR virus was according to the method of Talens and Zee.¹⁶ The virus suspension was 1st freed from cellular debris by centrifugation at 4 C for 15 minutes at 5,000 × g. The clarified virus was layered onto a cushion of 40% sucrose solution (w/v) and centrifuged for 1 hour at 65,000 × g. The pellet was layered onto a 10% to 40% (w/v) potassium tartrate gradient and centrifuged for 1 hour at 65,000 × g. Virus bands were diluted and pelleted for 90 minutes at 90,000 × g.

Extraction of Viral DNA—Viral DNA was isolated by the method of Minson et al.¹⁷ with minor modifications. Purified virus was mixed with sodium dodecyl sulphate (SDS) to a final concentration of 0.5%. Proteinase K,^b 40 µg/10 OD₂₈₀ of virus (optical density at 280 nm), was added, and the mixture was incubated at 37 C for 30 minutes. The solution was then extracted twice with phenol-chloroform (1:1) at 4 C followed by 1 extraction with chloroform-isoamylalcohol (24:1). The DNA was precipitated by the addition of 3 volumes of ethanol.

Nick Translation—The IBR-LA DNA was nick-translated in vitro, using the method described by Rigby et al.¹⁸ The reaction mixture contained the following ingredients: 1 µg of IBR-LA DNA, all 4 tritiated deoxyribonucleotide triphosphates (dATP, dCTP, dGTP, dTTP)^c at a concentration of 120 µCi/100 µl, and 1.2 U of DNA polymerase I and DNase I^e in a final volume of 100 µl. After incubation for 2 hours at 15 C, the mixture was extracted with phenol. The final aqueous phase was applied to a Sephadex G-75 column, and the DNA was eluted.

Preparation of Trigeminal Ganglia—Immediately after the calves were killed, the trigeminal ganglia were removed.¹⁵ They were embedded in 1% methylcellulose^d and stored at -70 C until further preparation. On the day of hybridization, the ganglia were sectioned (5 to 10 µm) with a cryostat-microtome onto glass slides previously dipped in a subbing solution containing 0.1% gelatin and 0.01% chrome alum (CrK(SO₄)₂ · 12H₂O). The tissue was fixed for 5 minutes in methanol-acetone (1:2) at -20 C, rinsed with 70%, 95%, and absolute ethanols and dried in air. Agarose (type II),^f 0.4%, was allowed to form a thin layer on the slide to prevent detachment of tissue. After drying overnight, the tissue was denatured by placing it in 0.05N NaOH at room temperature for 5 minutes and after several washings in ethanol (70%, 95%, absolute), it was dried in air.

In Situ Hybridization—The procedure for in situ hybridization was derived from various published methods¹⁹⁻²⁴. 30 to 50 ng (5 × 10⁵ cpm) of tritiated DNA was denatured by exposure to 100 C for 10 minutes. The SSC (1 × SSC = 0.15M NaCl, 0.015M Na citrate, pH 7.0) and formamide^e were added to give a final concentration of 2 × SSC and 46% formamide. This incubation mix was applied to each slide in a volume of 70 µl/slide and covered with a siliconized cover slip. Hybridization was performed in a sealed moist chamber containing Whatman's filter paper completely soaked in 2 × SSC and 46% formamide for 50 hours at 45 C.

The cover slip and the hybridization mix were removed by dipping the slide into 2 × SSC and 46% formamide at 40 C twice for 10 minutes. Nucleic acid bound unspecifically was removed by 3 additional washings in 2 × SSC at room temperature and one 5-minute wash in 2 × SSC and 5% trichloroacetic acid at 4 C. Finally, the slides were dipped twice each in 70%, 95%, and absolute ethanols before being air dried.

TABLE 1—Clinical Course of Experimentally Induced Latent Infection in Calves Inoculated with BHV-1 (LA Strain)

Calf No.	Virus recovery from nasal and ocular secretions on PID	Appearance of antibodies to BHV-1 on PID	Period of treatment with dexamethasone (PID)	Virus recovery after DM from nasal/ocular secretion on PID	Day killed (PID)	Antibody titer of calf when killed
1	3-9	12	None	...	30	1:50
2	5-14	14				1:50
3	5-14	14				1:452
4	2-9	12				1:100
5	12-14	19				1:56
6	3-14	(*)				1:13
7	7-14	19	55-59	60	103	1:20
8	5-7	12				1:13
9	3-7	14				1:63
10	2-16	(*)				1:13
11	2-9	12				1:403
12	2-9	14				1:806

* Calves with neutralizing antibodies to IBR virus before experimental infection.

Autoradiography^{20,25}—The dried slides were coated with autoradiographic emulsion^g by standard procedures. Completely dried slides were dipped in dioxan^e containing 1% PPO (2,5 di-phenyl-oxazol)^e and 0.2% POPOP (1,4-bis(5-phenyl-2-oxazolyl)-benzole).^e The autoradiographic exposure ranged from 72 to 120 hours at -70 C. The slides were then developed and stained with Giemsa stain before microscopic examination; 15 to 30 sections per ganglion were examined.

Results

Course of the Disease in Inoculated Calves—One day before they were inoculated, calves 1 through 12 were examined for the presence of IBR antibody and for the presence of virus in nasal and ocular secretions. The IBR virus was not detected in any of the calves, but calves 6 and 10 showed antibody titers of 1:32 and 1:50, respectively.

The clinical course of the disease in the calves was mild. There were no signs of disease, apart from slight nasal and ocular discharges, lasting for a few days. Virus was recovered from all calves for several days between PID 2 and 16. All seronegative calves showed seroconversion between PID 12 to 19. On PID 30, calves 1 through 10 were killed. Virus was not found in their nasal and/or ocular secretions at this time and the animals were still seropositive for IBR. (For details, see Table 1.)

Evidence for the Latency of the Experimental Infection—Calves 11 and 12 were given (iv) daily doses of DM (0.1 mg/kg of body weight) from PID 55 until PID 59. Virus shedding was observed from swabs taken on PID 60 (calf 11) and on PID 61 (calf 12). The animals showed no clinical signs of IBR. On PID 103 (corresponding to day 42 and 43 after the last detectable virus shedding), calves 11 and 12 were killed. Antibodies to BHV-1 were present in the serum, but virus was not detected (Table 1).

In situ Hybridization of Infected and Noninfected Cell Cultures—The specificity of in situ hybridization technique was demonstrated by comparing hybridization in IBR-virus infected and noninfected eBLC monolayers. In infected cells, silver grains, indicating the presence of IBR-viral DNA, were detected over the nucleus, as well as over the cytoplasm. The varying density of silver grains indicated a

^b Boehringer, Mannheim, Germany.

^c Amersham, Buckinghamshire, England.

^d Methocel MC 150, Fluka, Buchs, SG, Switzerland.

^e Fluka, Buchs, SG, Switzerland.

^f Sigma, München, Germany.

^g NTB-2, Kodak, Lausanne, Switzerland.

different state of viral reproduction in individual cells (Fig 1). Accumulation of silver grains was not observed in non-infected cells. The uniform distribution of silver grains in Figure 2 represents the background activity.

In Situ Hybridization of Trigeminal Ganglia—The IBR viral DNA was detected in the trigeminal ganglia of 9 latently infected calves in 13 of 23 examined ganglia. Of the sections examined, 4.9% (325 slides made out of 13 positive ganglia contained 16 cells with BHV-1 DNA) contained 1 cell with BHV-1 DNA. In 4 animals, viral DNA was found in

both trigeminal ganglia; in 3 animals, only in the left; and in 2, only in the right. Viral DNA was not detected in calves 2, 3, and 4, as well as in any section prepared from control calves (No. 1N through 6N). (For details, see Table 2.)

Specific hybridization, as evidenced by the accumulation of silver granules, was restricted to the nucleus of trigeminal ganglion cells. Neither in the cytoplasm of ganglion cells nor in any other cells of the ganglion tissue was IBR viral DNA present. In positive sections, only 1 or 2 neurons were found to contain an amount of viral DNA detectable by in situ hybridization (Fig 3A, B, C).

We found no correlation between the duration of virus excretion and the presence of viral DNA in the ganglia.

Discussion

The similarity between the pathogeneses of HSV-1 and BHV-1 infections is marked. After the primary inoculation, both viruses tend to establish a latent infection from which virus shedding with or without clinical signs of disease can be reactivated. With HSV-1, the site of latent infection has been shown to be the trigeminal ganglion where the DNA of the virus has been found in nerve cells.²⁶ A similar site and form of latency have been postulated for BHV-1.⁸

However, proof for this suggestion was lacking, and the fate of the virus between the disappearance of viral antigens and the reappearance of viral proteins in the nervous tissue shortly before recrudescence has not been documented.¹⁴ In the present study, we found that during the latent state, BHV-1 DNA is present in nuclei of neurons in the trigeminal ganglia. As in HSV-1 infection, only 4.9% of the sections contain viral DNA.²⁶

For the present experiment, it was essential to work with animals in which the virus was likely to have established a latent infection. To achieve this, the animals were inoculated intratracheally, and virus shedding and the serologic response to the virus were monitored throughout the experiment. Taking into account the results of Narita et al,²⁷ who have shown that no virus antigen was detectable at 18 days after inoculation, the animals were killed on PID 30. At that

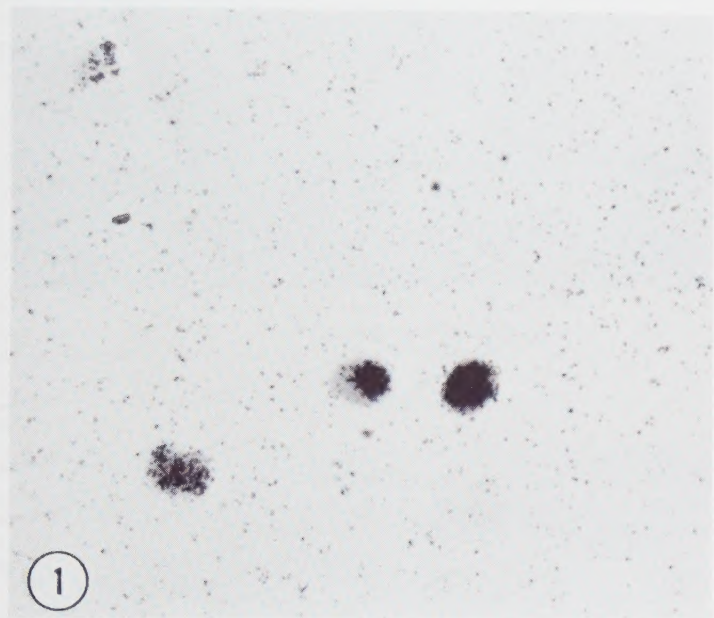


Fig 1—The BHV-1 infected embryonic bovine lung cells (eBLC) stained (Giemsa stain) after in situ hybridization. Accumulation of silver grains indicates synthesis of IBR viral DNA in the cells. The variation in granule density is caused by the different viral replication states in individual cells. × 250.

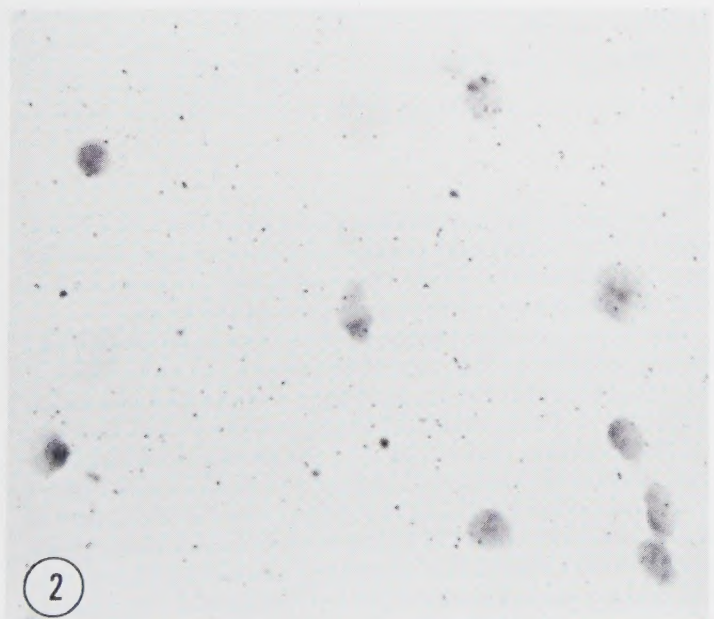


Fig 2—Noninfected eBLC (Giemsa stain), showing background activity with no accumulation of grains over cells, indicating absence of IBR viral DNA. × 250.

TABLE 2—Evidence for the Presence of BHV-1 DNA in Sections of Trigeminal Ganglia of Latent IBR-Infected Calves by in Situ Hybridization

Calf No.	Left ganglion	Right ganglion
INOCULATED CALVES		
1	Pos	Neg
2	Neg	Neg
3	ND	Neg
4	Neg	Neg
5	Pos	Pos
6	Pos	Pos
7	Pos	Neg
8	Pos	Pos
9	Pos	Neg
10	Neg	Pos
11	Neg	Pos
12	Pos	Pos
CONTROL CALVES (NONINOCULATED)		
1N	Neg	Neg
2N		
3N		
4N		
5N		
6N		

Pos = evidence for IBR viral DNA in 1 or more preparations; Neg = no evidence for any hybridization in any preparation made of this ganglion; ND = not done (the left ganglion of calf 3 was mutilated when the calf was killed, and further handling was not possible).

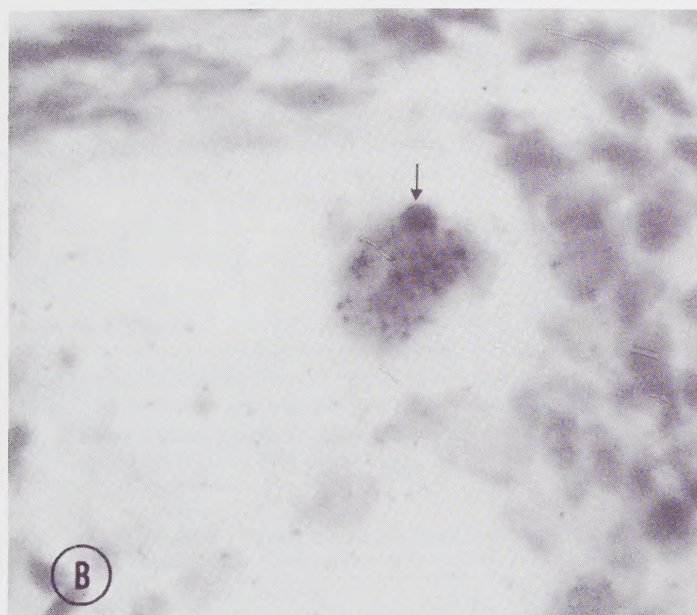
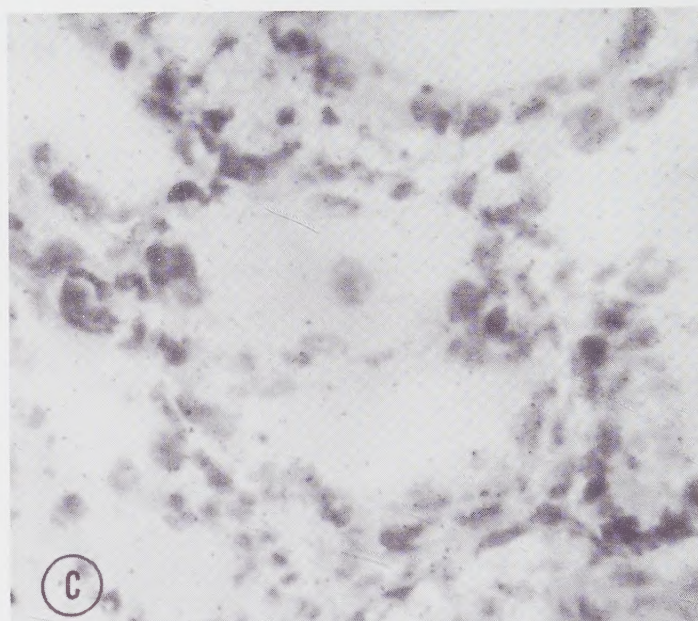
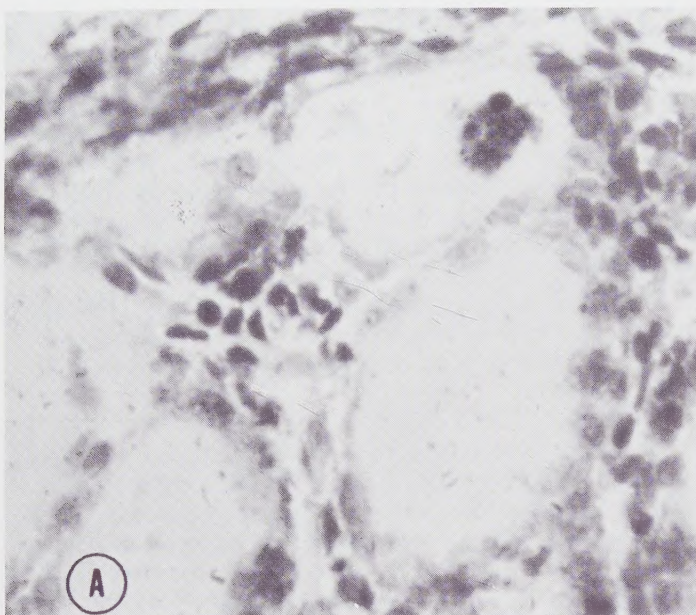


Fig 3—Localization of IBR DNA in a neuron cell of a bovine trigeminal ganglion. A, B, and C are preparations of the right ganglion of calf 10 exposed to autoradiography for 72 hours at -70°C . After development, the cells were stained with Giemsa.

A—Hybridization activity is seen to be strictly limited to the nucleus of the ganglion cell, indicating the presence of BHV-1 DNA. Background activity is evenly disseminated over the surrounding tissue and in the cytoplasm of ganglion cells. $\times 430$.

B—The same ganglion cell as in A, higher magnification. Giemsa stain has been reduced by use of a blue filter. The silver grains covering the nucleus have been accentuated by bringing the NTB-2 photographic emulsion layer into focus. Hybridization cannot be detected in the nucleolus (arrow). $\times 800$.

C—Nucleus of a ganglion cell and surrounding tissue showing no hybridization to BHV-1 DNA. $\times 430$. In this sectional plane, the nuclei of several neurons are not seen.

time, the animals were positive for antibody to BHV-1, indicating that the primary infection had taken place, whereas the absence of virus in nasal and ocular secretions argued against active virus replication. The latent state of infection was further substantiated by the stimulation of virus shedding following DM treatment.²⁸

The animals were seronegative before infection, except for calves 6 and 10. These 2 calves had antibody to BHV-1, indicating a former contact with a field strain of BHV-1. This contact may have led to the establishment of a latent infection. However, the presence of serum antibody to BHV-1 does not protect against superinfection with this virus.^{29,30} Moreover, different virus strains may establish a latent infection in the same calf.³¹ Preliminary experiments have shown that different BHV-1 virus strains are sufficiently homologous to lead to cross-hybridization.^h

^h Engels M, Institute of Virology, University of Zürich: Personal communication, 1980.

This opens the interesting possibility that the DNA found in the trigeminal ganglia of calves 6 and 10 could have originated from a field strain of BHV-1 virus, as well as from the strain used in the experimental infection.

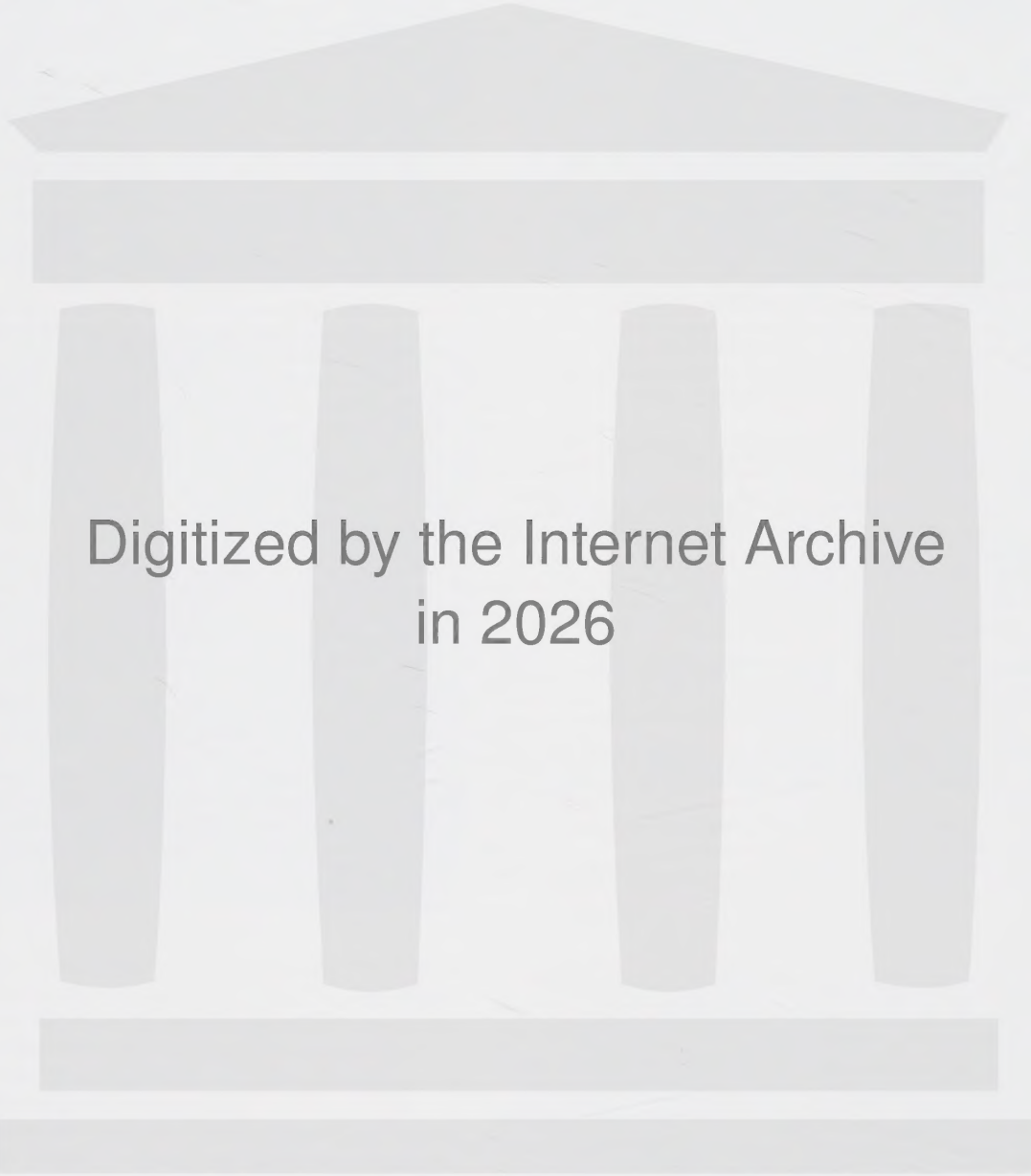
Having established that the calves were in all probability latently infected at the time of the search for viral DNA, it was equally important to show that the method chosen to locate BHV-1 DNA was sufficiently sensitive and specific. For this purpose, the conditions for in situ hybridization were optimized, using cell cultures infected with BHV-1 virus, and noninfected cell cultures served as the negative control. Hybridization to RNA was excluded by treating the sections with alkali before annealing. Cross-hybridization to other herpesviruses can be ruled out, because of the minimal homology among their DNA.³²

The BHV-1 infected eBLC showed specific hybridization over the entire cell body, indicating that viral DNA was present in the nucleus, as well as in the cytoplasm. This is

typical for cells containing actively replicating virus.¹⁹ In contrast, viral DNA detected in the trigeminal ganglia of latently infected calves was located in the nucleus of neurons only, indicating that the viral DNA in the trigeminal ganglia neurons was in an "inactive," nonreplicating form. The nuclear localization of viral DNA correlates with the apparent lack of antigen expression during the latent infection.^{14,27} As viral antigens must be accessible to antibody or cytotoxic lymphocytes if the infection is to be eliminated by the immune system, this indicates that the virus may escape immune surveillance as long as the latent state of the infection is maintained.

References

1. Miller NJ: Infectious necrotic rhinotracheitis of cattle. *J Am Vet Med Assoc* 126:463-467, 1955.
2. Armstrong JA, Pereira HG, Andrewes CH: Observation of the virus of infectious rhinotracheitis and its affinity with the herpesvirus group. *Virology* 14:276-285, 1961.
3. Bartha A, Hajdu G, Aldasy P, et al: Occurrence of encephalitis caused by IBR-virus in calves in Hungary. *Acta Vet Hung* 19: 145-151, 1969.
4. Hall WTK, Simmons GC, French EL, et al: The pathogenesis of encephalitis caused by the IBR-virus. *Aust Vet J* 42:229-237, 1966.
5. Bagust TJ, Clark L: Pathogenesis of meningoencephalitis produced in calves by IBR-herpesvirus. *J Comp Pathol* 82:375-384, 1972.
6. McKercher DG, Moulton JE, Madin SH, et al: Infectious bovine rhinotracheitis—A newly recognized virus disease of cattle. *Am J Vet Res* 18:246-256, 1957.
7. Snowdon WA: The IBR-IPV-virus: Reaction to infection and intermittent recovery of virus from experimentally infected cattle. *Aust Vet J* 41:135-142, 1965.
8. Davies DH, Duncan JR: The pathogenesis of recurrent infections with infectious bovine rhinotracheitis virus induced in calves by treatment with corticosteroids. *Cornell Vet* 64:340-366, 1974.
9. Brown SM, Subak-Sharpe JH, Warren KG, et al: Detection by complementation of defective or uninducible (herpes simplex type 1) virus genomes latent in human ganglia. *Proc Natl Acad Sci USA* 76: 2364-2368, 1979.
10. Cook ML, Stevens JG: Latent herpetic infections following experimental viraemia. *J Gen Virol* 31:75-80, 1976.
11. Baringer JR: Herpes simplex virus infection of nervous tissue in animals and man. *Prog Med Virol* 20:1-26, 1975.
12. Narita M, Inui S, Namba K, et al: Trigeminal ganglionitis and encephalitis in calves intranasally inoculated with IBR-virus. *J Comp Pathol* 86:93-100, 1976.
13. Babiuk LA, Rouse BT: Immune control of herpesvirus latency. *Can J Microbiol* 25:267-274, 1979.
14. Narita M, Inui S, Namba K, et al: Neural changes in recurrent infection of infectious bovine rhinotracheitis virus in calves treated with dexamethasone. *Am J Vet Res* 39:1399-1403, 1978.
15. Howman EJ, Easterday BC: Isolation of bovine herpesvirus-1 from trigeminal ganglia of clinically normal cattle. *Am J Vet Res* 41:1212-1213, 1980.
16. Talens LT, Yuan Chung Zee: Purification and buoyant density of infectious bovine rhinotracheitis virus (39159). *Proc Soc Exp Biol Med* 151:132-135, 1976.
17. Minson AC, Thouless ME, Eglin RP, et al: The detection of virus DNA sequences in a herpes type 2 transformed hamster cell line (333-8-9). *Int J Cancer* 17:493-500, 1976.
18. Rigby PWJ, Dieckmann M, Rhodes C, et al: Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J Mol Biol* 113:237-251, 1977.
19. Pagano JS, Huang ES: The application of RNA-DNA cytohybridization to viral diagnosis, in Kurstak E, Morisset R (ed): *Viral Immunodiagnosis*. New York, Academic Press Inc, 1974, chap 16.
20. Pardue ML, Gall JG: Nucleic acid hybridization to the DNA of cytological preparations, in Prescott DM (ed): *Methods in Cell Biology*. London, Academic Press Inc, 1975, vol 10, pp 1-16.
21. De Ley J, Tijtgat R: Evaluation of membrane filter methods for DNA-DNA hybridization. *Antonie Van Leeuwenhoek* 36:461-474, 1970.
22. Gillis M, De Ley J, De Cleene M: The determination of molecular weight of bacterial genome DNA from renaturation rates. *Eur J Biochem* 12:143-153, 1970.
23. McConaughy BL, Laird CD, McCarthy BJ: Nucleic acid reassociation in formamide. *Biochemistry* 8:3289-3294, 1969.
24. Plummer G, Goodheart CR, Henson D, et al: A comparative study of the DNA density and behavior in tissue cultures of fourteen different herpesviruses. *Virology* 39:134-137, 1969b.
25. Huang ES, Pagano JS: ³H cRNA-DNA cytohybridization in situ, in Kurstak E, Kurstak C (ed): *Comparative Diagnosis of Viral Disease*. New York, Academic Press Inc, 1977, I part A, pp 273-275.
26. Stevens JG: Latent characteristics of selected herpesviruses. *Adv Cancer Res* 26:227-238, 1978.
27. Narita M, Inui S, Namba K, et al: Neural changes in calves intravaginally inoculated with infectious bovine rhinotracheitis virus. *J Comp Pathol* 88:381-386, 1978a.
28. Gibbs EPJ, Pitzolis G, Lawman MJP: Use of corticosteroids to isolate IBR-virus from cattle in Cyprus after respiratory disease and ataxia. *Vet Rec* 96:464-466, 1975.
29. Sekizawa T, Openshaw H, Wohlenberg C, et al: Latency of herpes simplex virus in absence of neutralizing antibody: Model for reactivation. *Science* 210:1026-1028, 1980.
30. Darcel C le Q, Jericho KWF: Failure of a subunit bovine herpesvirus 1 vaccine to protect against experimental respiratory disease in calves. *Can J Comp Med* 45:87-91, 1981.
31. Nettleton PF, Sharp JM: Infectious bovine rhinotracheitis virus excretion after vaccination, short communications. *Vet Rec* 107:379, 1980.
32. Bronson DL, Graham BJ, Ludwig H, et al: Studies on the relatedness of herpes viruses through DNA-RNA hybridization. *Biochim Biophys Acta* 259:24-34, 1972.



Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41507201_0061

Am 15. November 1951 wurde ich in St. Gallen geboren.
In Herisau besuchte ich die Primarschule und während
eines Jahres die Sekundarschule.

1971 schloss ich an der Kantonsschule St. Gallen
mit der Matura Typ B ab. Anschliessend arbeitete ich
als Aushilfslehrer.

1972 - 1977 studierte ich an der Universität Zürich
Veterinärmedizin. Nach dem Staatsexamen arbeitete
ich als Assistent in der Praxis. Seit August 1979
bin ich am Institut für Virologie der Universität
Zürich.

